IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of :

Heribert SCHMITT-WILLICH et al. :

Serial No.: 08/319,357 : Group Art Unit: 2203

Filed: October 6, 1994 : Examiner: L. Chapman

For: DERIVATIZED DTPA COMPLEXES, PHARMACEUTICAL AGENTS CONTAINING THESE COMPOUNDS, THEIR USE, AND PROCESSES FOR THEIR PRODUCTION

DECLARATION UNDER 37 C.F.R. §1.132

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

SIR:

I, Gabriele Schuhmann-Giampieri, being duly warned declare that:

I am a citizen of Germany, residing at Marschnerstrasse 34, 12203 Berlin, Germany.

I possess the degree of Doctor of Natural Sciences, having studied Pharmacy at the Ludwig-Maximilians-Universitaet in Munich.

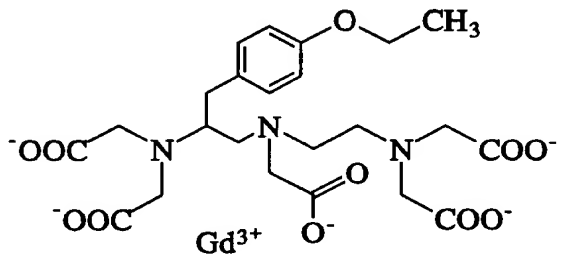
I am a member of the German Society of Pharmacology and Toxicology.

Since May 1988, I have been employed as a pharmacist by Schering, Aktiengesellschaft, Berlin, Germany, and am presently head of a research group for the pharmacology of contrast media.

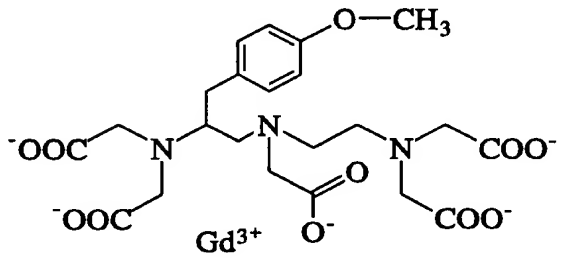
Under my supervision, excretion and relaxivity experiments were conducted for the chelate complexes gadolinium ethoxybenzyl DTPA (Gd-EOB) and gadolinium methoxybenzyl DTPA. Also, under my supervision, excretion experiments were conducted comparing the

biliary excretion of gadolinium methoxymethyl and Gd-EOB. The structures of these three chelate complexes are shown below:

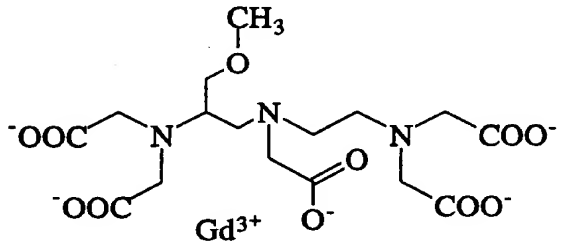
Gd-EOB



Gd-methoxybenzyl DTPA



Gd-methoxymethyl DTPA



Excretion

Using the technique of inductively coupled plasma atomic emission spectrometry (ICP-AES) which is very sensitive for the quantitative measurement of lanthanide ions including gadolinium (detection limit of 65 mmol Gd/L), the following excretion data were obtained in rats (Wistar-Han, 140-160 g).

Following single intravenous administration of 0.1 mmol Gd/kg body weight, biliary excretion of test substances was mea-

sured in anesthetized animals by collection of bile by catheterization of the bile duct up to 4 h after administration. Three animals per test substance were investigated. The mean cumulative biliary excretion of methoxybenzyl DTPA was 44.6% whereas in the case of EOB the mean cumulative biliary excretion was 73.9% of the administered dose.

Relaxivity

The T1 relaxation times of aqueous solution and of human blood plasma was measured with a Magnetic Resonance Spectrometer (Minispec, PC-20) operating at 0.47 T (20 MHz). An inversion-recovery pulse sequence was used for measuring the T1 relaxation rate. All measurements were conducted at 39-40°C, and at least three increasing concentrations of each test substance were measured beside blank samples.

Additionally, the gadolinium concentration of EOB and of methoxybenzyl-DTPA in the aqueous and in the plasma solution were measured and correlated with measured T1-relaxation rates by using standard least-square algorithm, thus allowing calculation of T1 relaxivity (slope of the function concentration versus relaxation rate). The T1-relaxivity of the methoxybenzyl-DTPA chelate complex was 4.54 L/mmol·sec in water and 6.89 L/mmol·sec in plasma, whereas the T1-relaxivity of the EOB chelate complex was 5.33 L/mmol·sec in water and 8.69 L/mmol·sec in plasma.

Excretion

Using the technique of inductively coupled plasma atomic emission spectrometry (ICP-AES) which is very sensitive for the quantitative measurement of lanthanide ions including gadolinium (detection limit of 65 mmol Gd/L), the following excretion data were obtained in rats (Wistar-Han, 140-160 g).

Following single intravenous administration of 0.1 mmol Gd/kg body weight, biliary excretion of test substances was measured in anesthetized animals by collection of bile by catheterization of the bile duct up to 4 h after administration. Three animals per test substance were investigated. The excretion test results are shown in the following table:

Excretion (% injected dose)	Gd-methoxymethyl DTPA	Gd-ethoxybenzyl DTPA
Urine 0-2 h	68.1	19.0
Urine 2-4 h	7.5	3.9
Urine Total	75.6	22.9
Bile 0-2 h	0.6	62.5
Bile 2-4 h	0.2	5.7
Bile Total	0.8	68.2
Liver 4 h p.i.	0.6	0.9
Total	77.0	91.8

As can be seen from the above, in the time period of 0-4 hours, less than 1% of the administered dose of Gd-methoxymethyl DTPA was excreted via the biliary system. Conversely, in the same time period, more than 68% of Gd-EOB was excreted by the biliary system. The biliary excretion of Gd-methoxymethyl DTPA is extremely low, not only in comparison to that of Gd-EOB but also to that of Gd-methoxybenzyl DTPA.

Thus, the higher biliary excretion of the EOB chelate complex together with the higher efficacy (higher relaxivity) demonstrates unexpected beneficial and advantageous results for using the EOB chelate complex in magnetic resonance imaging of the liver and the biliary system.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date

Gabriele Schuhmann-Giampieri

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